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# Using Phosphorus-Rich Biochars to Remediate Lead-Contaminated Soil: Influence on Soil Enzymes and Extractable P

Pacian Netherway <sup>1</sup>, Gabriel Gascó <sup>2</sup>, Ana Méndez <sup>3</sup>, Aravind Surapaneni <sup>4</sup>, Suzie Reichman <sup>1</sup> , Kalpit Shah <sup>1</sup> and Jorge Paz-Ferreiro <sup>1,\*</sup>

<sup>1</sup> School of Engineering, RMIT University, GPO Box 2476, Melbourne 3001, Australia; pacian.netherway@epa.vic.gov.au (P.N.); suzie.reichman@rmit.edu.au (S.R.); kalpit.shah@rmit.edu.au (K.S.)

<sup>2</sup> Department of Agricultural Production, Technical University of Madrid, Ciudad Universitaria, 28040 Madrid, Spain; gabriel.gasco@upm.es

<sup>3</sup> Department of Geological and Mining Engineering, Technical University of Madrid, 28040 Madrid, Spain; anamaria.mendez@upm.es

<sup>4</sup> South East Water Corporation, Waters Edge, 101 Wells Street, Frankston 3199, Australia; aravind.surapaneni@sew.com.au

\* Correspondence: jorge.paz-ferreiro@rmit.edu.au

Received: 29 February 2020; Accepted: 22 March 2020; Published: 25 March 2020



**Abstract:** In recent times, there has been increased focus on a holistic approach to soil remediation with consideration of social, economic and environmental factors. Consequently, there is a demand from practitioners and regulators alike for suitable ways to measure ancillary outcomes, for example, effects on soil quality. Here we show that biochar, when applied to land to remediate lead (Pb)-contaminated soils, can lead to environmental improvements not realized by adding mined or manufactured phosphates. Here, we study a Pb-contaminated soil amended with two phosphate fertilizers (slow- and fast-release) and with biochars produced from poultry litter and from biosolids at three temperatures (300 °C, 400 °C and 500 °C). The results show that, unlike the fast-release P fertilizer, biochars did not result in an increase in the amount of leachable P that could be released into the environment. Biochars prepared at 500 °C presented a higher value of the integrative geometric mean of soil enzyme activity, compared to the P fertilizers. Overall, our research shows that biochars, particularly those prepared at the higher temperature tested, are a suitable alternative to P fertilizers as an integrative remediation strategy in Pb-contaminated soils, enabling soil biological restoration.

**Keywords:** Biochar; soil quality; lead pollution; lead remediation; soil enzymes

## 1. Introduction

The release and build-up of lead (Pb) in soil affects urban communities all over the world. Humans can be exposed to Pb by eating food grown in impacted soils, by inhaling Pb-contaminated dust or by ingesting impacted soils. The risks of Pb to human health have been well documented, and the need to manage these risks remains ever-present [1]. Broadly, contamination risk mitigation strategies aim to break a source–pathway–receptor linkage. For example, dig and dump is a common approach involving the removal of the source of contamination by excavation and disposal at a landfill [2]. The pathway linkage can be broken by introducing a barrier between the contamination and the receptor, for example, by capping the area with clean soil or geotextile [3]. In situ chemical immobilization of Pb in soils is emerging as an attractive alternative [1]. This approach involves treating the soil with amendments, usually phosphorus-based, with the aim of transforming the Pb into a less soluble form. That is, when ingested with soil, the Pb may not be available for uptake into the blood

stream; therefore, the exposure pathway is limited. The technique is not without critics. Deployed amendments are generally mined or manufactured phosphorus, which are limited resources, while the runoff or leaching of excess P and soil acidification are poor environmental outcomes associated with the use of fast-release P fertilizer [1,4]. To this end, the use of biochar from biogenic waste sources of phosphorus is emerging as a promising alternative to conventional inorganic fertilizers [5].

Biochar has been increasingly used in the last few years for soil remediation. Mechanisms for metal immobilization have been studied profusely. The nature of the functional groups of the biochar [6], an increase in soil pH [7,8] and effects on water holding capacity [9] will affect remediation outcomes.

In view of the International Standard for Soil Quality—Sustainable Remediation, the effect of remediation on soil health is highlighted as an important metric of remediation performance [10]. One indicator of soil health is the activity of soil enzymes involved in important soil processes, for example, nutrient cycling and the mineralization of organic matter. The use of biological indices to evaluate soil quality is an approach being used more commonly in recent years, as they provide an early indication of changes in soil quality [11].

In this study, we measured the activity of soil enzymes following incubation with a range of phosphorus-rich biochars and manufactured phosphorus amendments (both slow- and fast-release) added to a Pb-contaminated soil. In a previous study, it was shown that these biochars performed comparably to conventional phosphorus amendments in reducing Pb bioaccessibility [5]. However, a more holistic approach to remediation should also consider the impacts on other soil properties. Consequently, in the present article, we study the effects of several treatments used for the remediation of a Pb-contaminated soil on soil enzyme activity and available P.

The activity of soil enzymes has been used multiple times as an early indication of the effects of management changes in soil quality, as they change more rapidly than physical or chemical properties [11]. Several indices have been developed for the daunting task of evaluating changes in soil quality in a holistic manner. The number of biological properties considered in an index has been variable. Some studies have reported the possibility of estimating changes in soil quality based on three biochemical properties. This has been done following two main approaches. Puglisi et al. [12] developed a linear index using three soil enzymes. On the other hand, the geometric mean of soil enzyme activity (GMea), using a variable number of enzymes in the index, has been used in several instances as an early indication of changes in soil quality following the addition of amendments. In particular, some authors report the effect of biochar on GMea using three soil enzymes [13].

Consequently, we hypothesized that the feasibility of using biochar over manufactured amendments could be enhanced in view of ancillary environmental improvements.

## 2. Materials and Methods

The soil and biochars used in this experiment have been described in detail in a previous article [5]. The properties of the soil and the biochars used are included in the Supplementary Table S1. Sieved soils (< 2 mm) were incubated in 250 mL polyethylene jars, with each jar containing 300 g of soil. The control was the polluted, untreated soil. The remaining treatments comprised poultry litter (PL) and biosolids (BS) biochar prepared at 300 °C, 400 °C and 500 °C (PL300, PL400, PL500, BS300, BS400, BS500), monocalcium phosphate (MCP) (Sigma-Aldrich) and NTS Softrock™ (NTS) (nutri-tech.com.au) added to the soils at 1% w/w. Soils were incubated at 60% water holding capacity (WHC) using ultrapure water (18 MΩ.cm). The moisture was replenished every two days by the addition of ultrapure water. Each of the eight treatments plus the untreated control were replicated four times.

After three months of incubation, a subsample was collected and the activity of three soil enzymes were measured according to the methods described in a previous article [14]. Acid phosphomonoesterase, β-Glucosidase and arylsulphatase activities were determined by measuring the amount of p-nitrophenol released during enzymatic hydrolysis after incubation at 37 °C. Acid phosphomonoesterase, β-glucosidase and arylsulphatase used respectively 16 mM p-nitrophenyl phosphate, 25 mM p-nitrophenyl-β-d-glucopyranoside and 5 mM p-nitrophenyl sulphate as substrates.

Four mL of buffer were added and then the samples were incubated. The buffer solution was Modified Universal Buffer for phosphomonoesterase and  $\beta$ -glucosidase while acetate buffer (pH 5.8) was used for arylsulphatase. The incubation time was 30 min for phosphomonoesterase and one hour for both,  $\beta$ -glucosidase and arylsulphatase. Four mL of 2 M  $\text{CaCl}_2$  was added (to stop the reaction and to avoid the brown coloration caused by organic matter) and the liberated p-nitrophenol was extracted with 0.2 M NaOH for phosphomonoesterase. For  $\beta$ -Glucosidase the p-nitrophenol released was extracted with THAM-NaOH at pH 12. The p-nitrophenol released during enzymatic hydrolysis was determined using a spectrophotometer at a wavelength of 400 nm. To account for the amounts of adsorbed p-nitrophenol, a standard curve was prepared for each treatment, as per previous works [14,15]. The activity of each of these three enzymes was expressed as  $\mu\text{mol p-nitrophenol g}^{-1} \text{h}^{-1}$ .

The geometric mean of enzyme activity (*Gmea*) has been used in several instances as an indicator of soil quality in response to the addition of amendments as it has been shown to be sensitive to land use changes [15,16]. *Gmea* was calculated as follows:

$$Gmea = (Glu \times Phos \times Aryl)^{1/3} \quad (1)$$

where, *Glu*, *Phos* and *Aryl* are  $\beta$ -Glucosidase, phosphomonoesterase and arylsulphatase, respectively.

Analysis for Olsen-P was completed by ALS Laboratories (NATA Accredited) according to [17].

Statistical differences between treatments were calculated using SPSS version 18.0. A one-way ANOVA was conducted in order to see the various treatment effects. Means were considered to be significantly different when  $p < 0.05$  using Tukey's test. A principal component analysis (PCA) was carried out for soil enzymes and selected biochar properties (pH, cation exchange capacity (CEC), surface area, H/C ratio and P content).

### 3. Results and Discussion

The results of the enzyme assays are presented in Table 1. Phosphomonoesterase is involved in the cycling of P from phosphomonoesters to plant-available inorganic P. Previous studies have observed that the activity of this enzyme should increase in response to a shortage of P and vice versa [14,18]. Phosphomonoesterase activity increased following the addition of PL500, BS300, BS400, BS500 and NTS, whereas it was inhibited following the addition of PL300 and MCP and was unaffected by the addition of PL400. Notably, MCP (a large source of readily available P) decreased the activity of phosphomonoesterase by more than 50% compared to the control. The application of PL300 led to a similar decrease, whilst PL400 had no effect, likely reflecting the availability of P in these treatments, specifically, an increase in pyrolysis temperature has been shown to decrease the availability of P [19].

The activity of  $\beta$ -glucosidase was reduced or unaffected following treatment; notably, the largest decrease was observed in MCP, followed by NTS, PL400 and BS400. This is consistent with previous results [15]. Interestingly, biochars prepared at 400 °C led to a larger decrease in  $\beta$ -glucosidase compared to those prepared at 300 °C and 500 °C. Inherent differences in the biochars, for example, hydrophobicity, surface area and pore size, could also be involved in this effect [20,21], although these mechanisms are poorly understood.

Contrary to previous results [15], the application of biochar to soil affected the activity of arylsulphatase, an enzyme involved in the catalysis of ester sulfate bonds. The activity of arylsulphatase was similar to  $\beta$ -glucosidase, with the largest decrease in activity being observed for MCP. Most of the treatments resulting in lower  $\beta$ -glucosidase values also resulted in lower arylsulphatase values. This was the case for PL300, PL400, BS400, MCP and NTS. BS500 increased the activity of this enzyme compared to the control, which could be due to the depletion of organic carbon in the biochars produced at 500 °C, whereby the activity of this enzyme is known to be inhibited by the addition of organic amendments [18].

**Table 1.** Soil enzyme assay and Olsen P results. Data for enzyme activity are in  $\mu\text{mol p-nitrophenol g}^{-1} \text{h}^{-1}$ . Different letters within the same column indicate that the data are statistically significant ( $p < 0.05$ , Tukey's test).

Sample	Phosphomonoesterase	$\beta$ -Glucosidase	Arylsulfatase	GMea	Olsen P (mg/kg)
Control	4.56 $\pm$ 0.09c	1.21 $\pm$ 0.10e	0.17 $\pm$ 0.02de	0.99 $\pm$ 0.03ef	14 $\pm$ 2a
PL300	2.87 $\pm$ 0.10b	1.03 $\pm$ 0.05cd	0.13 $\pm$ 0.02bc	0.74 $\pm$ 0.05b	17 $\pm$ 3a
PL400	4.53 $\pm$ 0.44c	0.94 $\pm$ 0.09bc	0.11 $\pm$ 0.01b	0.76 $\pm$ 0.04bc	23 $\pm$ 2a
PL500	5.66 $\pm$ 0.15de	1.15 $\pm$ 0.11cde	0.21 $\pm$ 0.01f	1.12 $\pm$ 0.03g	24 $\pm$ 1a
BS300	5.24 $\pm$ 0.16d	1.16 $\pm$ 0.05cde	0.15 $\pm$ 0.01cd	0.97 $\pm$ 0.01de	17 $\pm$ 2a
BS400	5.92 $\pm$ 0.14ef	0.83 $\pm$ 0.08b	0.12 $\pm$ 0.01b	0.84 $\pm$ 0.05bc	18 $\pm$ 2a
BS500	6.47 $\pm$ 0.28e	1.09 $\pm$ 0.01cde	0.19 $\pm$ 0.02ef	1.09 $\pm$ 0.05fg	16 $\pm$ 2a
MCP	2.02 $\pm$ 0.10a	0.65 $\pm$ 0.04a	0.08 $\pm$ 0.01a	0.46 $\pm$ 0.02a	170 $\pm$ 23b
NTS	7.23 $\pm$ 0.51f	0.83 $\pm$ 0.04b	0.12 $\pm$ 0.01b	0.86 $\pm$ 0.10cd	16 $\pm$ 1a

There is no paucity of literature stating that it is not possible to use a single enzyme as an indicator of soil quality. See, for example, the study by Trasar-Cepeda, which found that individual soil enzymes do not possess the ability to differentiate different levels of pollution in a systematic manner [22]. In fact, enzymes could respond positively to the increased concentration of a pollutant. Consequently, as indices of enzyme activities are more reliable when discussing pollution remediation studies, we calculated the GMea. In our study, the addition of MCP led to a 53% reduction in GMea, whilst PL300, PL400, BS400 and NTS also led to a decrease in GMea, though to a lesser degree, ranging from 13% to 25%. Notably, the biochars prepared at 500 °C (PL500 and BS500) led to an increase in GMea by 10% and 13%, respectively, which is relatively consistent with previous studies. For example, in the study by Paz-Ferreiro, biochar produced from sewage sludge at 600 °C led to a 19% increase in GMea compared to the control [15].

No correlation between any single biochar property and soil enzymes was found (data not shown). A PCA was undertaken in order to obtain more insights into the factors driving soil enzyme activity for the samples amended with biochar (see Table 2). The first two axes of the PCA plot accounted for 80% of the data variability (51% of the variability was explained by the first axis and 29% of the variability was explained by the second axis). Enzyme activities, together with surface area, had the highest loading in the second factor, suggesting that the increased pore area available to soil microorganisms for colonization had a positive effect on soil enzyme activity. Overall, soil enzyme activity is altered by the temperature of pyrolysis and the length of pyrolysis time [23], but considerable uncertainties prevail about how changes in biochar properties brought on by these factors impact on soil enzymatic activity.

**Table 2.** Factor analysis of enzyme activities in the treatments with biochar.

Property	Factor 1	Factor 2
pH	0.992	−0.051
CEC	0.955	−0.075
Surface area	0.227	0.696
H/C ratio	−0.954	−0.121
Total P	0.992	−0.060
Phosphomonoesterase	−0.446	0.786
$\beta$ -glucosidase	0.195	0.606
Arylsulfatase	0.157	0.892

As mentioned previously, excessive P leaching is an undesirable environmental outcome associated with the immobilization of Pb through the use of P fertilizers. In our study, MCP led to an order of magnitude increase in the amount of Olsen P ( $173 \pm 23$  mg/kg) compared to the untreated control and the remaining treatments. This has a number of implications for the wider ecology and for soil remediation. Establishing a successful plant cover is imperative to reduce the eolic transport of polluted soil particles. However, large amounts of available P can be toxic to plants, for example, those in the Proteaceae family [24,25]. Moreover, in the particular geographic context of this study, an Olsen extractable P concentration of around 20 mg/kg can be lethal to the seedlings of P-sensitive Australian native species [26]. When undertaking a remediation options assessment, a holistic approach should consider potential impacts on the wider ecology. In this way, for example, the deployment of MCP to immobilize Pb in an urban parkland in Australia could present a risk to native plant species. With respect to leaching and runoff of excess P, a study found that when the Olsen P concentration exceeded 54 mg/kg, the amount of P released into water increased markedly from 4.0 mg/kg to 28 mg/kg [27]. The addition of MCP increased the Olsen P to  $170 \pm 23$  mg/kg from a value in the control polluted soil of  $14 \pm 2$ . Whilst assessing a change point was not within the scope of this study, there was a potential for the MCP treatment to result in excessive P leaching. With respect to the biochars and NTS amendments, the treatments did not lead to a statistically significant change in Olsen P, which aligns with previous studies reporting that P-rich biochars tend to act as a slow-release source of P [19,28].

To our knowledge, in terms of contamination remediation, this is the first study to assess the effect on soil quality of adding conventional P amendments compared with P-rich biochars. This is important given that these biochars have been shown to perform comparably to conventional options for the purposes of immobilizing Pb in soil [5].

#### 4. Conclusions

In sum, the application of an amendment for the purposes of immobilizing heavy metals in soil should consider wider environmental outcomes to ensure that a net environmental benefit is achieved. In this study, we found that the application of MCP, a conventional amendment used to remediate Pb-contaminated soils, reduced the activity of soil enzymes involved in important biogeochemical soil processes. Secondly, we observed that the application of MCP has the potential to result in excess P being released into the environment. Our hypothesis was that biochars produced from poultry litter and biosolids could present a better alternative. The results indicate that the application of biochars produced from these feedstocks at 500 °C maintained or increased the activity of soil enzymes, signaling an increase in soil quality. Our results indicate that P-rich biochars do not affect the availability of P in the receiving soil. This indicates that the release of excess P into the environment, associated with Pb remediation, could be avoided by substituting phosphorus fertilizers with P-rich biochars.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/10/4/454/s1>, Table S1: Soil and biochar characteristics.

**Author Contributions:** P.N. and J.P.-F. conceived the experiment. P.N. collected and analyzed the data. P.N., G.G., A.M., A.S., S.R., K.S. and J.P.-F. wrote the article. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Additional Information:** The authors have no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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